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BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDIES OF HYDRA. I. ANALYSIS OF CONTRACTION PULSE PATTERNS.[1]

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A characteristic feature of the behavior of some hydroid polyps is that their spontaneous activity consists of rhythmically re-occuring behavioral events. Examples are: the concerted oral movements of the tentacles followed by peristalsis of the proboscis in *Tubularia* (Josephson and Mackie, 1965); hydranth contraction of *Obelia* (Morin, 1969); stalk contraction in isolated polyps of *Corymorpha* (Rushforth, unpublished observations); and contraction of the body column and tentacles in *Hydra* (Passano and McCullough, 1963). In these four hydroids electrical potentials are associated with the periodic movements. The potentials are relatively large, on the order of several millivolts when externally recorded.

Large spontaneous potentials and associated periodic movements, however, are not universal in hydroids. Neither rhythmic behavior nor endogenous events have been observed in *Pennaria* (Fred Delcomyn, University of Oregon, personal communication) and *Hydractinia* (Darrell Stokes, University of Hawaii, personal communication). In *Cordylophora,* although very slow rhythmic behavior occurs (Fulton, 1963), the animal is electrically silent unless fed or stimulated (Mackie, 1968). At the present time, no hypotheses have been proposed to explain the existence of these divergent classes of hydroid behavior.

The behavior and electrical activity in Hydra

Passano and McCullough (1962) were the first investigators to record electrical correlates of spontaneous behavior in *Hydra.* In a series of papers they described the properties of two endogenously active coördinating systems in this animal (Passano and McCullough, 1964, 1965; McCullough, 1965).

One system termed the Rhythmic Potential System, produces pulses (RP's) rhythmically with frequencies ranging from 1 to 10 per minute. These pulses are small, relatively rapid, compound potentials on the order of tens of microvolts when recorded externally. While potential RP pacemakers are located throughout the whole column, RP's usually begin in the basal region of the animal. The pulses are conducted in the column at about 4 cm per sec. Such rhythmic potentials trigger gastrodermal muscle contraction, causing elongation of the body column (Shibley, 1969).

A second coördinating system produces large, slow potentials when recorded with external electrodes. The pulses are of up to 500 msecs in duration and have amplitudes up to several tens of millivolts. The system was designated the Contraction Burst System by Passano and McCullough (1964), since the pulses

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were associated with contraction of the epidermal musculature and occurred usually in bursts. On the basis of long-term recordings (lasting 10 to 12 hours) from five species of hydra, Rushforth (1966) found that in many animals the electrical pulses and body contractions occur primarily as single, widely spaced events rather than in bursts. In view of this finding the potentials were termed Contraction Pulses (CP's) rather than Contraction Burst Potentials. This term has been used subsequently by Rushforth (1967a) and others (Josephson, 1967, Josephson and Macklin, 1967, 1969). In retrospect, it would have been more appropriate to have called these potentials Column Contraction Pulses to distinguish them from contraction pulses recorded from the tentacles of the animal (Rushforth, 1967b). However, in order not to add another term to the rapidly proliferating nomenclature of pulses recorded from hydroids, the term Contraction Pulses (CP's) will be retained in the present study. The pulses are equivalent to those designated as Contraction Burst Potentials by Passano and his co-workers.

The present study is a quantitative description of the temporal patterns of Contraction Pulses in two species of *Hydra:* the large Belgian species *H. pirardi* Brien and the North American variety *H. pseudoligactis.* It is likely that *H. pseudoligactis* is synonymous with *H. canadensis* which deserves priority (Adshead, Mackie and Paetkau, 1963). However, the animals have been cultured for several years as *H. pseudoligactis* and this name will be continued to be used until it can be shown that the species is the same as that Rowan described as *H. canadensis* (Rowan, 1930). These species were chosen since they represent the extremes on a continuum of behavior and electrical activity of ten species of hydra thus far investigated. The contraction pulse activity of *H. pirardi* consists predominately of bursts of potentials while that of *H. pseudoligactis* consists almost exclusively of single events. Under conditions of external stimulation, however, the electrical activity of the latter species may be changed to give a temporal pattern of bursts of potentials similar to that of *H. pirardi.*

This investigation is a first step in an effort to construct testable models for the production of potentials by pacemaker systems in hydroids. The current study was undertaken to quantify the characteristics of inter-pulse intervals in two selected species of *Hydra.* The characteristics of the interval measurements will be compared in subsequent studies with those observed for other hydroids, and with those of intervals generated using theoretical models.

Materials and Methods

The conditions employed for culturing the hydra were similar to those described by Loomis and Lenhoff (1956). Animals were raised in a medium consisting of glass distilled water to which 1.5×10^{-5} M $CaCl_2$, 1.2×10^{-3} M $NaHCO_3$, and 1.2×10^{-4} M Na_4 EDTA had been added. The hydra were fed daily with a plentiful supply of freshly hatched *Artemia salina* nauplii. The temperature was held at 21 ± 1° C and the animals were reared in constant illumination, approximately 30 foot candles.

The electrical recordings were from hydra randomly selected from the culture dishes 24 hours after feeding. Continuous recordings of the spontaneous electrical activity of individual hydra were made using a glass suction electrode (Josephson, 1967). The individual recording periods were 10 to 12 hours in duration. The electrode has a bell-shaped opening 0.4–0.6 mm in internal diameter about 1 mm from the open end. The other end of the electrode is attached to a syringe by means of flexible tygon tubing and is held in a micromanipulator. The hydra is lightly sucked into the open end of the electrode, and sits with its base in the bell-shaped opening (Fig. 1). The electrode is filled with culture solution and contains a silver wire inserted through the tubing to increase the conductivity. Electrical contact in this system is made between the tissue of the hydra base, the culture medium and the silver wire. A reference electrode consists of a coil of silver wire approximately 5 cm from the recording electrode. Electrical potentials from the preparation are amplified using capacitor-coupled amplifiers having long time constants, with the final display on an oscilloscope and penwriter. Both electrodes are placed in 200 ml of culture solution in a finger bowl. The recording electrode is positioned so that the hydra touches neither the bottom of the dish nor the upper surface film with its tentacles.

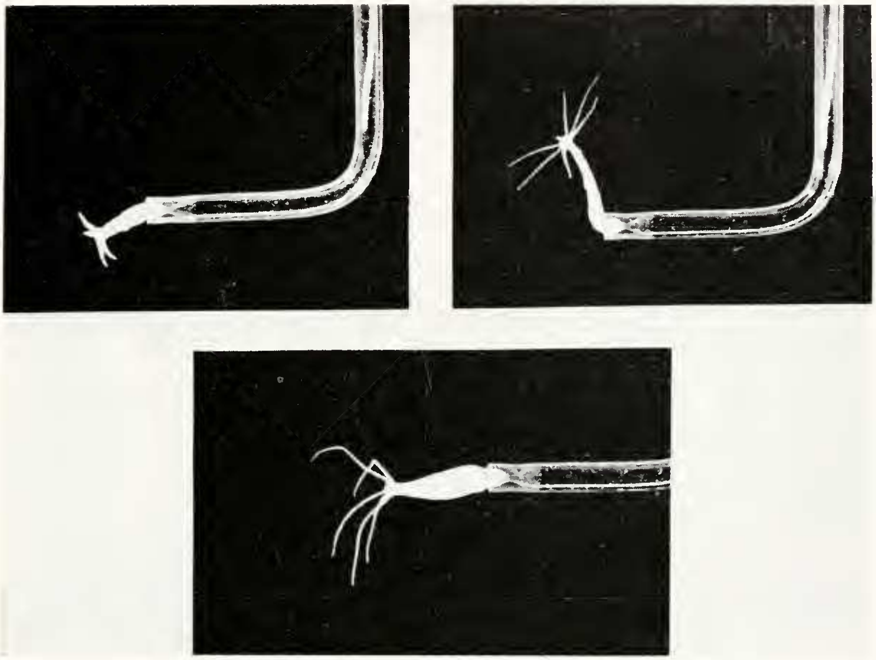

FIGURE 1. Hydra placed in the recording electrode: upper left: *H. pirardi* in a contracted state; upper right: *H. pirardi* in an elongated position; lower: *H. pseudoligactis* in a normal relaxed position.

It was found that increased suction on the base of the hydra in the electrode affected both the frequency and temporal patterning of pacemaker activity. Therefore, during the recording periods the suction was kept as low as seemed feasible. Occasionally because of the light suction on the electrode, the hydra would pull its base out of the electrode during the recording period. It was found possible to feed hydra attached to the electrode and have them produce buds over several days while still recording their pacemaker activities.

Visual observations using a dissecting microscope were made of the overt behavior of the hydra while simultaneously monitoring its electrical activity. Column contractions were manually recorded during phases of the electrical recordings using a telegraph key which activated a channel of the penwriter. Such visual observations were made at the start and termination of a 12 hour record of electrical activity to ensure correspondence between the recorded pulses designated as CP's and column contractions of the animal.

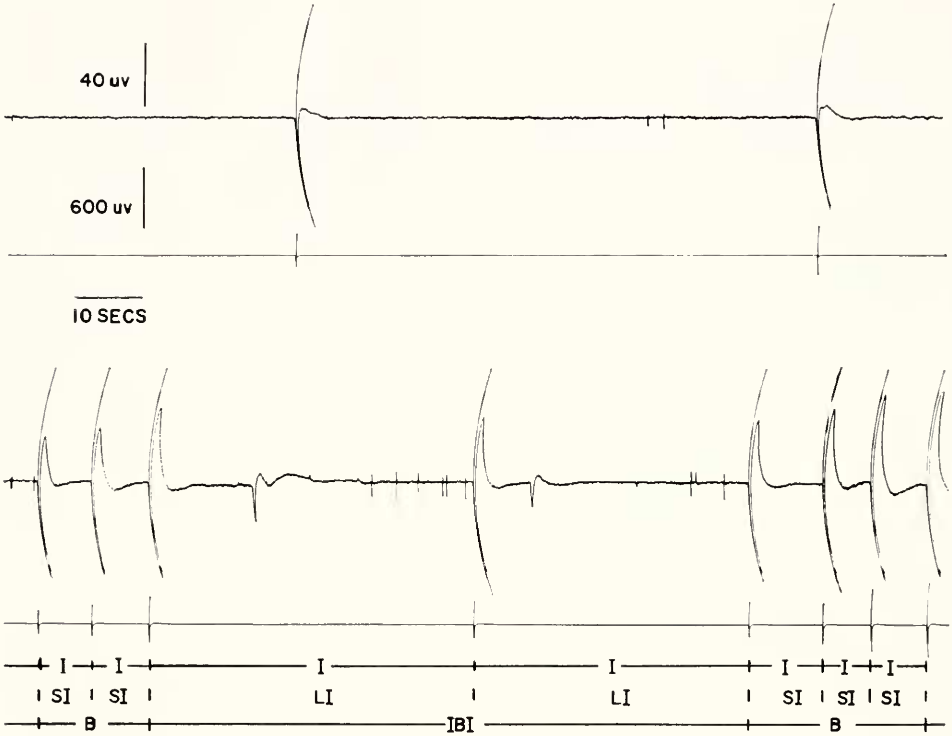

Figure 2. Electrical records of hydra. Upper two records: *H. pseudoligactis*, lower two records: *H. Pirardi*, for definition of the symbols see the text. The positive polarity is in the upward direction in this and other figures of electrical recordings.

In order to distinguish between the behavioral event of column contraction and the associated electrical event the following terms are used: (1) a column contraction designates a single contraction of the body column which may be visually observed, or recorded using a transducer as described by Josephson (1967); (2) a contraction burst is a behavioral event consisting of a series of individual column contractions, each contraction in the series separated by less than 20 seconds from a subsequent or preceding column contraction in the burst. At the termination of a contraction burst the hydra appears as a tight ball with its column fully contracted; and (3) a Column Contraction Pulse (designated here as a Contraction Pulse, or CP) is a single electrical potential preceding a column contraction by 0.1 to 0.2 seconds. The electrical correlation of a contraction burst is a burst of Contraction Pulses (a CP burst).

Measurements were made from the penwriter records of the intercontraction pulse interval lengths to an accuracy of 0.4 seconds. Both inter-pulse interval histograms and joint interval scatter diagrams were used to characterize the distributions of the inter-pulse intervals. These methods are described later in the results section.

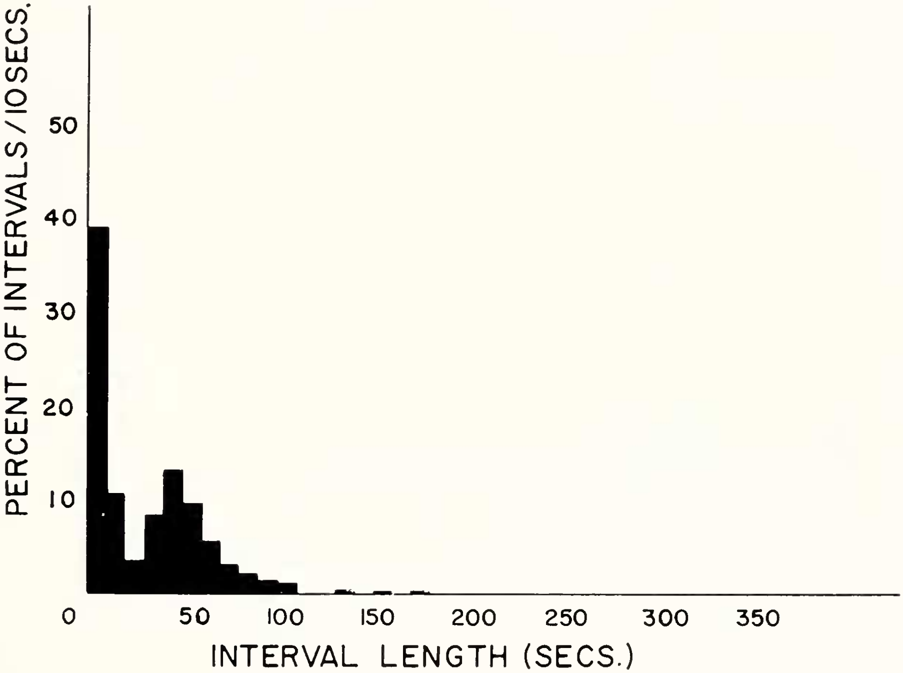

FIGURE 3. Inter-pulse interval histogram for *H. pirardi*. The intervals are from a 10 hour record from a single animal.

With *H. pseudoligactis*, the CP system was sometimes induced to fire predominately in bursts rather than as single CP's by: (1) increasing the electrode suction; (2) giving intermittant light stimulation; or (3) exposing the animal to live *Artemia salina* nauplii. The CP pattern was determined for three different levels of suction: 0, 10, 20 cm of water, as measured by a manometer attached to the tygon tubing of the recording electrode. Intermittant light stimulation consisted of successive exposures of the hydra to two minutes of "strong light" and two minute periods of ambient light. The "strong light" was supplied by an American Optical Lamp, Model 353 which housed a G. E. bulb, model 1493, operated at 6 volts. The bulb was placed 15 cm from the preparation and light was directed through a heat filter and the culture solution onto the whole hydra. The CP activity was monitored during 1½ hour period of intermittant light stimulation and compared with that for a similar period following the termination of such stimulation. Contraction Pulses were recorded from unstimulated hydra and their temporal patterns compared with those attained after the animal had fed to satiation on artemia. The nauplii were introduced one at a time into the medium above the hydra, creating minimal mechanical disturbance to the preparation.

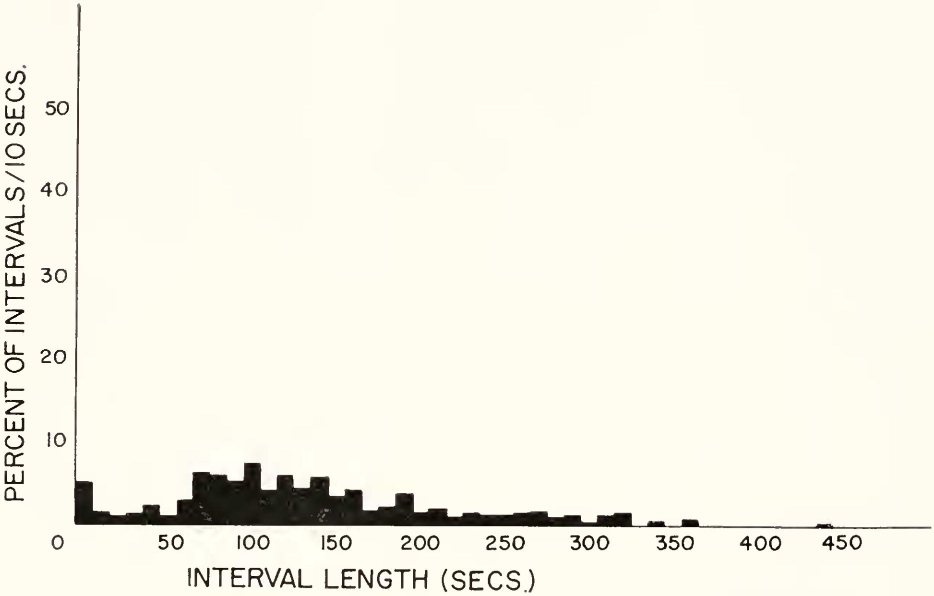

FIGURE 4. Inter-pulse interval histogram for *H. pseudoligactis.* The intervals are from a 10 hour record from a single animal.

RESULTS

Temporal patterns of spontaneous Contraction Pulses

Inter-species differences. The temporal patterns of spontaneous Contraction Pulses were markedly different in the two species. Figure 2 shows a 140 second portion of a continuous record from a single *H. pseudoligactis* (upper pair of records), and a *H. pirardi* (lower pair of records). The upper channel of both pairs of records has greater sensitivity and was used to record both the CP's which are the large potentials, as well as Rhythmic Potentials (RP's) and Tentacle Contraction Pulses (TCP's) which are much smaller potentials. These smaller pulses are clearly evident in the record of *H. pirardi.* At the sensitivity used to record RP's and TCP's considerable truncation of the CP amplitude occurs in the penwriter records. Thus, the second channel was used at a lower sensitivity to record undistorted CP's unaccompanied by the smaller potentials.

H. pseudoligactis gave CP's predominantly as single widely spaced events. While *H. pirardi* gave both single pulses and bursts of potentials. When the interval lengths between successive CP's for all intervals in a 10 hour record were plotted in the form of a percentage histogram, the resulting distribution was clearly bimodal in the case of *H. pirardi* (Fig. 3). The lower modal class, consisting of approximately 40 per cent of the intervals for this preparation, represents short intervals occurring within bursts, while the upper modal class (about 13 per cent of the intervals) represents longer intervals not in the bursts. The trough between the two modes was used as a criterion to classify intervals into long and short intervals in the electrical recordings. On the basis of such histograms it was found that 20 seconds was a suitable cut-off value to characterize intervals as belonging to the long or short class. A burst was defined as consisting of two or more successive pulses separated by short intervals (SI). An inter-burst interval (IBI) was defined as the sum of all long inter-

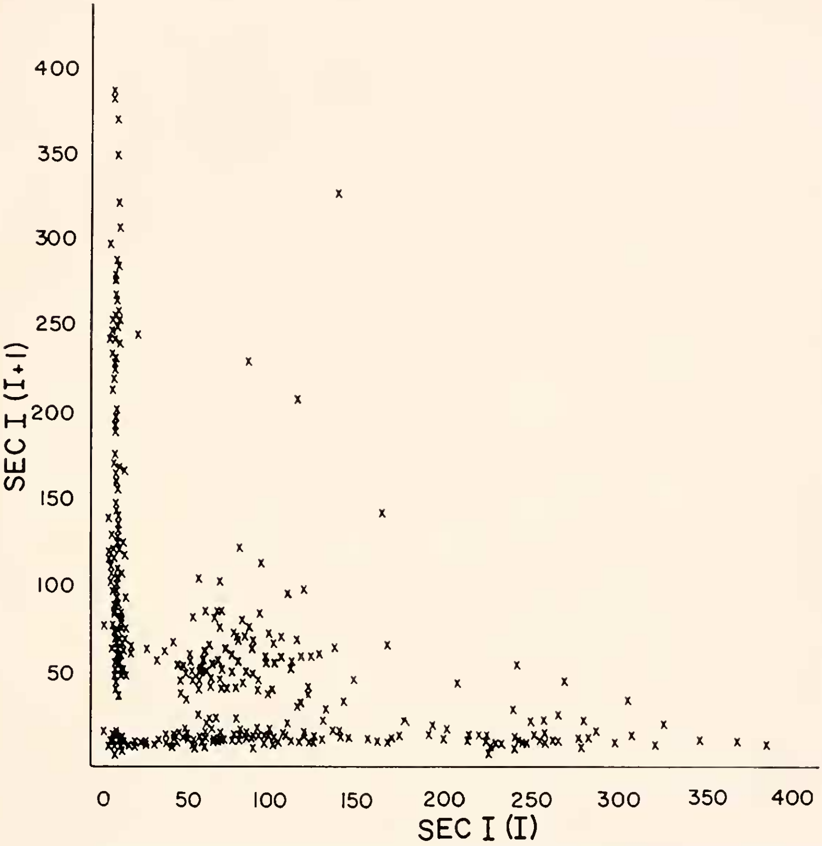

FIGURE 5. First order joint interval scatter diagram for *H. pirardi*. For further details see text. The preparation is the same as that for Figure 3.

vals (I.I) between two adjacent bursts. The segment of the *H. pirardi* record depicted in Figure 2 consists of seven inter-pulse intervals, five of which are short intervals while the remaining two are long intervals. The record contains two bursts and one inter-burst interval.

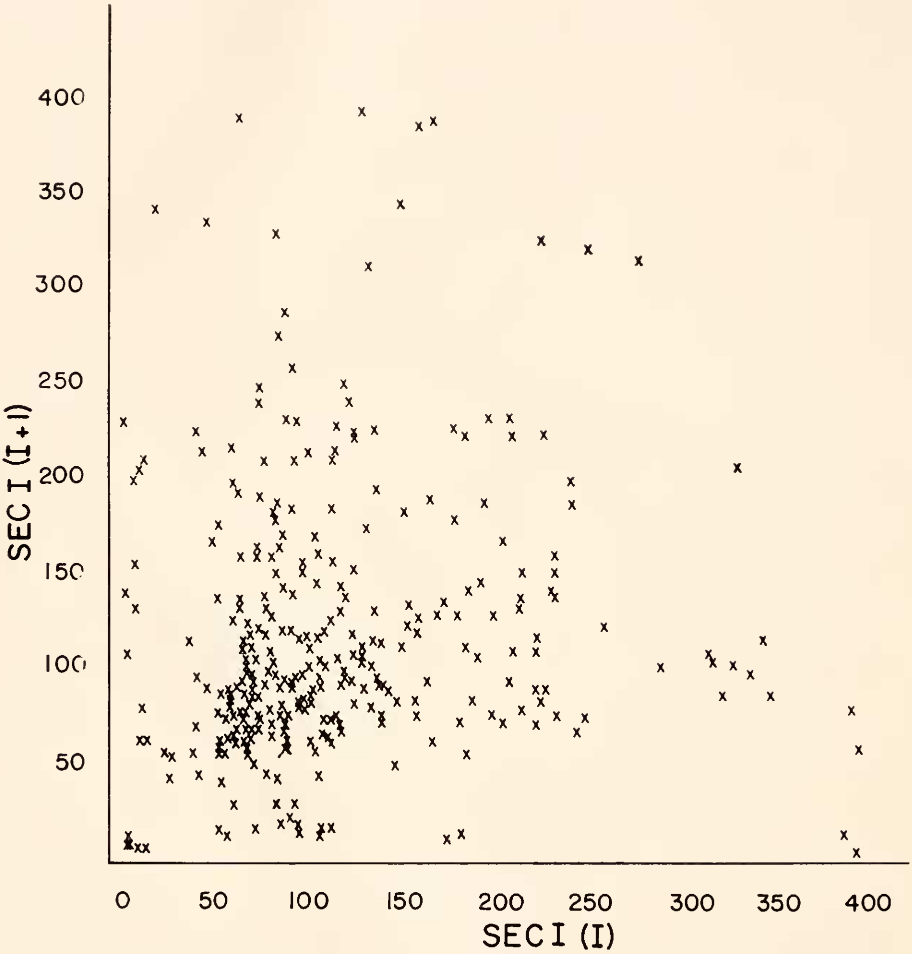

FIGURE 6. First order joint interval scatter diagram for *H. pseudoligactis*. For further details see text. The preparation is the same as that for Figure 4.

In contrast to the histograms for *H. pirardi* which contained a large percentage of short intervals (about 50 per cent for the preparation shown in Figure 3), those of *H. pseudoligactis* did not show bimodality and had few short intervals. The inter-pulse histogram for all intervals in a 10 hour recording period for the *H. pseudoligactis* preparation previously described, is plotted in Figure 4. In this case only 9 per cent of the intervals are short intervals (*i.e.*, less than 20 secs) indicating that there are few bursts for this animal.

The temporal patterns of CP's for the two species were compared using a second graphical procedure, a joint interval scatter diagram. This method has been used to quantitatively describe the spontaneous activity of a single neuron (Rodiech, Kiang and Gerstein, 1962). In Figure 5 the first order joint interval scatter diagram is plotted for the *H. pirardi* preparation. This is a graph of each successive inter-CP interval plotted as the ordinate measure against the inter-CP interval of the preceding interval as the abscissa. Thus the i + 1th interval is plotted against the ith interval, giving a graph of $n - 1$ points from a record of n inter-CP intervals. As is seen for this preparation, records containing a large number of bursts give characteristic L-shaped plots. Points close to the origin constitute intervals between pulses within a burst. Those near the abscissa and parallel to it are intervals which precede bursts; those near to the ordinate depict intervals following a burst. In contrast to this graph, the first order joint interval scatter diagrams for the *H. pseudoligactis* shows points widely dispersed and roughly symmetrically distributed about 45° line through the origin (Fig. 6). The graphs for all ten preparations of *H. pseudoligactis* were quite similar to that shown here, and were quite different from the L-shaped plots characterizing the burst patterns of the ten specimens of *H. pirardi*.

TABLE I

Measurements of intervals between Contraction Pulses for H. pirardi *and* H. pseudoligactis. *The data are from 10–12 hour recordings for 10 hydra of each species*

Measurement	Mean (Standard error)		
	H. pirardi	*H. pseudoligactis*	
Number of intervals/12 hr	1167 (137)	397 (30)	$P < 0.001$
Short interval (sec)	9.0 (0.9)	6.3 (0.1)	$P < 0.05$
Long interval (sec)	94 (14)	128 (8)	$P < 0.05$
Per cent of intervals classified as long intervals	42 (4)	89 (3)	$P < 0.001$
Number of bursts/12 hr	279 (29)	26 (6)	$P < 0.001$
Number of intervals/burst	2.5 (0.3)	1.7 (0.1)	$P < 0.05$
Mean interval/burst (sec)	9.3 (0.9)	7.0 (1.0)	$P > 0.10$
Burst lengths (sec)	20.7 (1.4)	10.8 (1.1)	$P < 0.001$
Inter-burst interval (sec)	151 (20)	1746 (336)	$P < 0.001$

The temporal patterns of the contraction potential records from the two species were further compared with measurements utilizing long and short intervals and the derived quantities of bursts and inter-bursts. A summary of these measurements from the ten hydra of the two species is given in Table I. Mean values together with their standard errors are presented for nine parameters of Contraction Pulse activity. It is evident from this table that the overall frequency of CP's, as measured by the number of intervals/12 hours is much greater for *H. pirardi*. This is a result of a greater number of bursts and therefore a smaller inter-burst interval length than for *H. pseudoligactis*.

When the burst characteristics of the two species are compared it is noted that the burst lengths of *H. pirardi* are longer. This results from both a larger number of intervals per burst and a greater mean interval per burst for this species. Differences in the distributions for burst and inter-burst characteristics were investigated in order to determine statistical measures which most clearly distinguished between the two species. It is seen from Figure 7 that two measurements, the number of bursts/12 hours and the burst length may be used to separate animals of the two species into non-overlapping sets. Thus, we see that for these two species the temporal characteristics of the column contraction pacemaker potentials might be used as criteria for taxonomic classification.

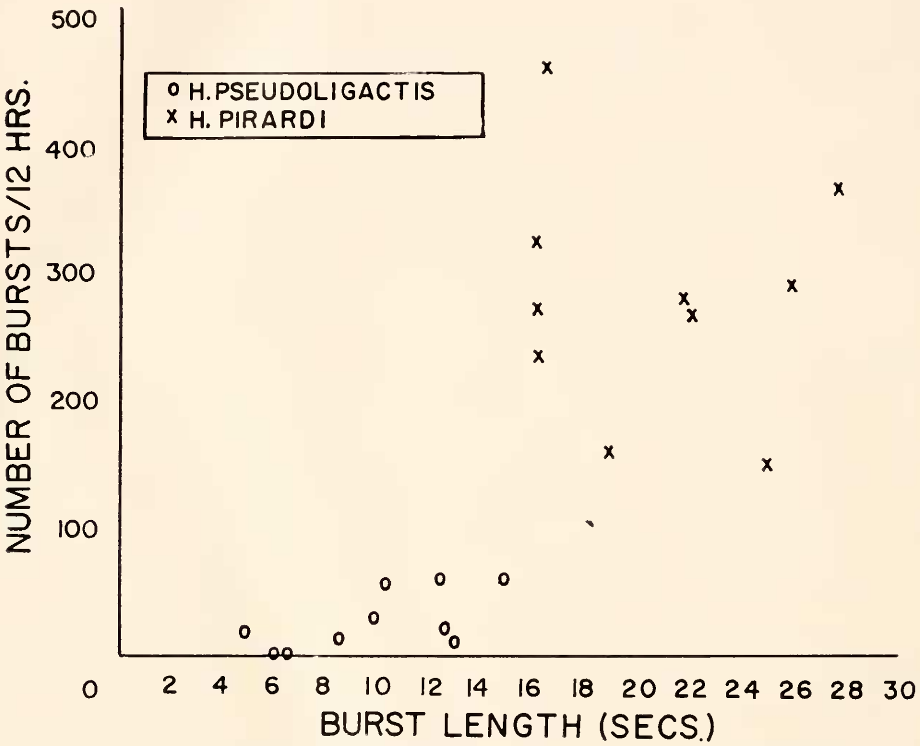

Figure 7. Graph of the number of brusts/12 hours and the burst length for ten preparations of *H. pseudoligactis* (open circles) and *H. pirardi* (crosses).

Characteristics of bursts of contraction pulses: (a) Amplitudes of CP's within bursts. Measurements were made of some additional characteristics of the bursts of *H. pirardi.* There were insufficient bursts in the records of *H. pseudoligactis* to apply statistical analyses. However, the general relationships observed for *H. pirardi,* appear to apply to the records of spontaneously produced CP bursts in *H. pseudoligactis,* and are quite evident when animals of this species are stimulated to give bursts of potentials.

An easily discernible feature of bursts of potentials is the increase in the amplitudes of successive CP's in a burst. Both Passano and McCullough (1964) and

Josephson (1967) noted that CP's are compound potentials consisting of both positive and negative phases when recorded externally. In the present study the pulses contain both positive and negative phases (and also an after potential due to capacitor discharge in the capacitor-coupled recording system). The initial positive and negative components were measured in the long-term records. In preparations of *H. pirardi* the amplitudes of both the negative and positive components of the potentials were found to increase for successive pulses in a burst. Except for one of the ten preparations studied, in which the amplitudes of the first two pulses in a burst were approximately the same, a successive increase in the amplitude of CP's in a burst was a constant feature of the records. In Table II, the relationship of the CP amplitudes and the position of the pulse in a group of CP's is given for the *H. pirardi* preparation previously described. Analysis of variance tests indicated that the increase in both phases of CP's within a burst is statistically significant ($P < 0.05$).

TABLE II

Amplitude of a CP and position of the CP in a group of CP's for H. pirardi. *The data are from a 12 hour recording from a single animal*

Number of pulses per group	Mean amplitude (mv): Positive phase / Negative phase — Position of pulse in the group: 1	2	3	4	Number of groups
1	1.93 1.44				96
2	1.77 1.46	2.49 1.80			87
3	1.81 1.42	2.53 1.76	3.18 2.44		49
4	1.88 0.82	2.53 2.06	3.17 2.90	3.64 3.53	27

Characteristics of bursts of contraction pulses: (b) Lengths of short intervals within a burst and long intervals within an inter-burst. Passano and McCullough (1964) observed that spontaneous CP bursts in *H. pirardi* lasted less than 30 seconds and consisted of from 5 to 8 pulses. They noted that the inter-CP interval lengths in a burst were shorter at the mid-point of the burst than either at the start or end of the burst.

Analyses were made of the inter-pulse intervals in recordings of *H. pirardi*, to determine both the interval lengths of short intervals within a burst, and the lengths of long intervals within an inter-burst interval. Data were pooled for 10 animals and the mean lengths were determined for intervals at various positions in a burst and in an inter-burst. The relationship between the interval length and the position of the interval in a burst is given for bursts of various sizes

TABLE III

Interval length and position of interval in a burst for H. pirardi. *The data are based on 10–12 hour recordings of each of 10 individual animals*

Number of short intervals per burst	Mean interval (sec)					Number of bursts
	Position of interval in the burst					
	1	2	3	4	5	
1	10.4					860
2	10.1	9.8				789
3	9.5	7.4	8.5			597
4	8.5	6.0	5.7	6.9		392
5	8.7	5.7	5.2	5.5	6.9	302

in Table III. Although in our recordings of *H. pirardi* the burst lengths were smaller (about 20 seconds, see Table I) and consisted of fewer pulses (2 to 5 usually), the characteristic pattern of interval lengths within a burst observed by Passano and McCullough (1964) was evident. Interval lengths were shortest in mid-burst than in the early stages or at the termination of a burst. The length of the first short interval was significantly greater ($P < 0.001$) than the lengths of other intervals in the burst.

Table IV reveals the relationship between the length of a long interval and its position in the inter-burst interval. The first long interval is significantly longer ($P < 0.001$) than the subsequent long intervals in the inter-burst interval. However, there is a systematic decrease in the lengths of progressive intervals in the inter-burst interval. Thus, the temporal pattern of short intervals within a burst is quite different from that of long intervals within an inter-burst interval.

Modifications of CP patterns in H. pseudoligactis *induced by external stimulation*

The previous results indicate that in *H. pirardi,* there are normally many spontaneous bursts of CP's contrasted with *H. pseudoligactis* in which bursts of CP's are rare events in unstimulated preparations. Any models devised for pacemaker

TABLE IV

Interval length and position of interval in an inter-burst interval for H. pirardi. *The data are from 10–12 hour recordings for each of 10 individual animals*

Number of long intervals per inter-burst interval	IBI (sec)	Position of interval in the inter-burst interval					Number of inter-burst intervals
		1	2	3	4	5	
1	106	106					1595
2	145	93	52				983
3	205	86	66	53			246
4	244	78	59	62	45		77
5	356	88	74	70	66	58	27

activity in *Hydra* should include provisions for both types of pulse patterns. *H. pseudoligactis* will produce bursts when appropriately stimulated. Effective stimuli include: (1) increased suction of the hydra in the recording electrode; (2) intermittant light stimulation of the whole preparation; and (3) exposure to stimuli inducing a feeding reaction in the animal. When *H. pseudoligactis* is induced to give bursts of CP's, their temporal patterns are similar to those previously noted for preparations of *H. pirardi.*

(1) *The effect of increased electrode suction.* In the course of long-term recordings of the spontaneous activity of several species of *Hydra,* it was observed that animals damaged during placement in the electrode produced many RP's. McCullough (1965) also noted an increased RP frequency in hydra placed in stressful recording situations. For animals such as *H. pirardi,* which normally produce many bursts of CP's, the initial effect is an elongation of the inter-CP burst interval accompanied by more pulses per burst and longer burst lengths. On the other hand, for hydra usually giving single CP's, such as *H. pseudoligactis,* the effect is to change the CP pattern to one primarly of burst of potentials.

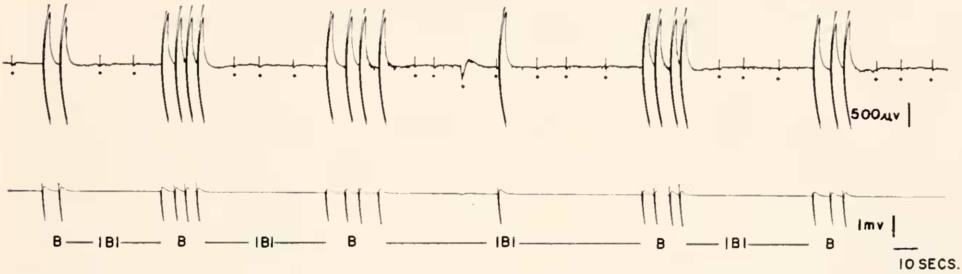

FIGURE 8. Electrical record of *H. pseudoligactis* under high electrode suction (20 cm of water).

In order to study the induction of bursts in *H. pseudoligactis* more systematically preparations were exposed to three different levels of suction in the recording electrode (0, 10, 20 cm of water). It was found that either intermediate (10 cm) or high (20 cm) pressure induced bursts of CP's accompanied by enhanced RP frequencies during inter-CP burst intervals. An example of an electrical record of a preparation at the higher pressure is given in Figure 8. Both the CP bursts and single RP's are easily discernible in the upper channel recording, and contrast markedly with the records characteristic of unstimulated *H. pseudoligactis* (see upper records of Figure 2).

In Table V, nine measurements of inter-CP intervals are compared for the three pressure regimes. As the suction on the base of the hydra is increased there is a significant increase in the frequency of CP bursts ($P < 0.01$), and therefore a marked reduction in the inter-burst interval length. There is significant decrease in the per cent of intervals which are long intervals, and also a reduction in the long interval length ($P < 0.01$). However, while increased pressure results in more short intervals within a burst ($P < 0.05$), the mean interval per burst is not affected significantly ($P > 0.10$).

CP bursts in *H. pseudoligactis* induced by increased electrode suction have the same characteristics as those of unstimulated *H. pirardi:* (1) both positive and negative phases of the CP amplitudes increase progressively with successive pulses in a burst; (2) intervals are shorter in mid-burst than at the start or end of the burst; and (3) long intervals significantly decrease in length with successive positions in the inter-burst interval.

(2) *The effect of intermittant light stimulation.* Several studies have shown that the effect of light on *Hydra* is complex, giving rise to both inhibition and excitation of pacemaker systems (Passano and McCullough, 1962, 1964, 1965; Rushforth, 1967a). When *H. pirardi* is stimulated with light for two minutes (about 600 ft-c), followed by two minute periods of ambient light (about 30 ft-c), the CP pattern is altered. The normal burst pattern is changed to one in which bursts occur exclusively during the two minute periods of direct illumination (Rushforth, 1967a). In contrast when preparations of *H. pseudoligactis* are exposed to this regime, CP's are inhibited during light stimulation. On direct illumination of this species, the frequencies of both RP's and TCP's are increased. During the interposed periods of ambient light, however, the CP System is induced to fire in bursts. For a sample of 80 periods of illumination, the CP frequency was significantly smaller (0.15 ± 0.04 versus 5.45 ± 0.17, $P < .01$) and the RP frequency significantly greater (7.15 ± 0.19 *versus* 1.34 ± 0.14, $P < 0.01$) compared with control periods of ambient light.

TABLE V

Effects of pressure on CP interval measurements in H. pseudoligactis. *The data are based on a one hour recording at each pressure for each of eight animals*

Measurement	Mean (Standard error)		
	Low pressure (0 cm)	Intermediate pressure (10 cm)	High pressure (20 cm)
Number of intervals/12 hr	260 (37)	438 (59)	930 (376)
Short interval lengths (sec)	9.4 (1.2)	9.7 (5.8)	11.0 (0.7)
Long interval lengths (sec)	201 (28)	160 (27)	130 (29)
Per cent of intervals classified as long intervals	94 (8)	61 (7)	36 (10)
Number of bursts/12 hr	10 (4)	62 (7)	165 (82)
Number of intervals/burst	1.2 (0.2)	2.0 (0.5)	1.8 (0.4)
Mean interval length/burst (sec)	10.4 (1.3)	10.8 (0.8)	11.3 (0.7)
Burst lengths (sec)	11.7 (2.4)	18.1 (3.0)	20.5 (5.3)
Inter-burst interval lengths (sec)	4653 (1095)	655 (97)	272 (99)

An analysis of the stimulated bursts in the ambient light period showed the short interval lengths depended on their positions in the burst in a similar manner to spontaneously produced bursts in *H. pirardi*, or bursts in *H. pseudoligactis* stimulated by increased electrode suction. The intervals are shorter in mid-burst.

(3) *Effects of feeding.* In previous studies of the contraction behavior of various species of *Hydra*, it was observed that feeding stimuli inhibited spontaneous contractions and those induced by external stimuli (Rushforth, 1967a). Subsequent experiments in which both the contraction behavior and correlated electrical activity were simultaneously monitored showed that following CP inhibition during

feeding of an *Artemia* or exposure to the tripeptide reduced glutathione the CP frequency was increased significantly before returning to prestimulus levels. During a period of approximately 1 hour of increased CP activity, the temporal pattern of electrical pulses of *H. pseudoligactis* were quantified by comparing interpulse measurements taken before and after artemia ingestion. After feeding there are relatively fewer long intervals and considerably more bursts than before exposure to *Artemia*, for comparable time periods. There is a significant reduction both in the long and short interval lengths ($P < 0.01$), while the number of pulses per burst is significantly increased after ingestion of the artemia ($P < 0.01$).

The bursts induced by feeding have the same general features as those elicited by light or by increased electrode suction, or as those spontaneously produced by *H. pirardi*. Short intervals are shortest in mid-burst, long intervals decrease systematically within an inter-burst, and the amplitudes of pulses increase in size either throughout the burst or in the initial part of the burst levelling off to a constant value in the late stages.

Discussion

Passano and McCullough (1964) described several characteristics of the pacemaker system of coordinating periodic contractions of the body column in *Hydra* in some detail. In observations of three species (*H. littoralis, H. pirardi,* and *H. carnea*) they emphasized that electrical output of this system consisted primarily of bursts of potentials. However, they noted that single contractions, causing only a slight shortening of the animal, comprise approximately 10 per cent of the hydra's contraction behavior. In the current study the temporal pattern of burst of potentials for undisturbed species of *H. pirardi* has been contrasted with the pattern found in *H. pseudoligactis*. In this latter species unstimulated hydra give primarily single, widely spaced pulses and only occasionally produce bursts of potentials. However, when these animals are stimulated, the temporal pattern is changed to one largely consisting of bursts.

Three features of the interval patterns were characteristic for unstimulated *H. pirardi* and for *H. pseudoligactis*, induced to produce bursts: (1) amplitudes of pulses increased with successive positions in a burst; (2) intervals were shorter in mid-burst than at the start or termination of the burst; and (3) intervals decreased in length with successive positions in the inter-burst interval. These three features have been observed in other species of *Hydra* (Passano and McCullough, 1964, Rushforth, unpublished) and appear inherent properties of the temporal CP pattern in this coelenterate.

In studies of the electrical properties of the body wall of *H. oligactis*, Josephson and Macklin (1967, 1969) indicate that CP's are generated by the epithelial cells. They suggest that the inner membranes of the ectodermal epitheliomuscular cells are sites of CP production. Strumwasser (1967) suggested that the process generating periodic bursts of spikes in a neuron could be explained on the basis of an internal oscillator. "The frequency of his internal oscillator determines the burst frequency, whereas the interaction between the amplitude of the oscillator and a fixed discharge threshold determines the length of the burst and the number of spikes in it" (Strumwasser, 1967, page 306). Thus long intervals (between bursts) and short intervals (within bursts) in *Aplysia* are thought to result from a single generating mechanism.

In *Hydra*, the interval lengths of long intervals were found to have quite a different functional relationship to their position in an inter-burst interval from that of short intervals within bursts. Both in unstimulated preparations of *H. pirardi*, and in *H. pseudoligactis* induced to give bursts of potentials by increased electrode suction the intervals decrease successively in length throughout the inter-burst interval. The interval immediately following a burst was significantly longer than the subsequent intervals prior to the next burst. The different patterns suggest that different mechanisms seem to be involved in the production of short and long intervals.

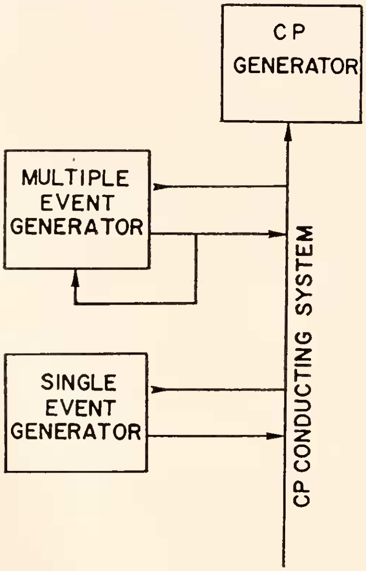

FIGURE 9. Schematic model of the CP system of *Hydra*. An arrow indicates excitatory input, a reverse arrow indicates inhibitory input.

The following model is offered as one which could account for the observed CP patterns. The proposed model is shown schematically in Figure 9. There are two elements, one producing single events and another producing multiple events. Both elements trigger the CP conducting system and thus initiate CP's. The multiple event generator has short term excitation to itself, such excitation leads to firing in bursts, and is also responsible for the decrease of interval lengths in the initial phases of the burst. There is also inhibitory feedback to the multiple event generator, probably mediated *via* the CP conducting system. This inhibitory feedback is slow rising, and the inhibitory effects of several CP's in succession are accumulative. The growing inhibition within a burst leads to increased intervals toward the end of the burst and eventually the termination of the burst. The joint effect of the excitatory and inhibitory components gives rise to the observed pattern of intervals within a burst, *i.e.* intervals are shorter in mid-burst than at the start or end of the burst.

The inhibitory effect of single CP's upon the multiple event generator can be directly demonstrated by driving the CP System with single stimuli. Exciting the CP System once each 15 to 30 seconds with single electric shocks or pulses of mechanical stimuli inhibit CP bursts (Passano and McCullough, 1964, Rushforth and Burke, in preparation). Indirect evidence for the inhibition of CP bursts by CP singles may be derived from an analysis of inter-CP intervals in long-term records consisting of both single pulses and bursts. The inter-burst interval is significantly longer ($P < 0.01$) if it includes a CP single than if no CP is present in the interval. With increasing numbers of single CP's the inter-CP burst interval is correspondingly increased in length (see Table IV).

It is proposed that there is inhibitory feedback to the single event generator mediated by the CP conducting system. Such inhibition results in the decrease in the intervals between single CP's following a burst of CP's throughout the inter-burst interval. This suggests gradual escape of the single event generator from long lasting inhibition following a CP burst.

The model proposed for the CP System is incomplete. Modifications of the model are necessary to take into account interactions of the CP System with both the Rhythmic Pulse System and the Tentacle Pulse System. The model is likely to be further modified to take into account the effects of external stimuli on the pacemaker system. Photic, mechanical and chemical stimuli are likely to have complex influences on the pacemaker systems, possibly affecting more than one component.

A major problem, as with all other hydroids, is the identification of the cellular elements involved in the pacemaker and conduction activities. Passano and McCullough (1965) proposed that the pacemaker system for CP's was the ectodermal nerve net. The size of the CP's, however, seemed too large to be exclusively the result of activity from the small dispersed neurons in the nerve net. Josephson and Macklin (1967, 1969) demonstrated that *Hydra* has a transepithelial potential. The fluid in the body cavity is typically 15 to 40 mv positive with respect to the outer bathing solution. Superimposed on this transepithelial potential are the negative going (depolarizing) CP's. It was hoped initially that direct measurement of the transepithelial potential and possible triggering of CP's in response to depolarizing current pulses might provide direct measurements for the construction of models for the production of CP's. However, CP's were found to have several surprising features in relation to the transepithelial potential which made such efforts unfeasible. Josephson and Macklin (1969) found: (1) the amplitude and frequency of CP's are essentially independent of the transepithelial potential when the latter is altered by imposed current; (2) there is little change in the impedance of the hydra column during CP firing; (3) when the transepithelial potential is clamped at zero, CP's continue to appear spontaneously as current spikes. There are four membranes between the inside of the body cavity and the outside solution, only one of which is likely to be the CP-generating membrane. Josephson and Macklin have shown that the CP-generating membrane forms only a small fraction of the total transverse impedance of the column. This unfortunately means that the CP-generating membrane cannot adequately be studied by measuring potentials across the body wall, or passing current through it, since the presence of large series impedance elements masks the properties of the CP-generating membrane.

The mechanisms by which CP's are conducted along the hydra column are unknown. Activity in the nerve net may trigger epithelial cells to produce these large electrical potentials. However, nerve free epithelia capable of producing propagated electrical pulses have been found in hydrozoans (Mackie, 1965, Mackie and Passano, 1968), so that it is possible that CP conduction as well as initiation is due to properties of the epithelial cells. In the present study the same characteristics of contraction potentials have been investigated in two species of *Hydra*. These characteristics impose boundary conditions on the parameters for models of pulse production. In subsequent papers we will investigate the properties of isolated hydroid pacemaker systems and the interactions of such pacemakers in the intact animal.

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SUMMARY

1. The temporal patterns of spontaneously produced Contraction Pulses (CP's) were investigated in two species of *Hydra*. It was found that *H. pseudoligactis* frequently give CP's as single, widely spaced events, whereas, *H. pirardi* produce many CP bursts. Inter-pulse interval histograms and joint order scatter diagrams, constructed on the basis of 10 to 12 hour recordings from 10 preparations of the two species, were employed to depict these contrasting temporal patterns of pulse production. The burst frequency and the burst length for CP's were found to be so different for the two species that these measurements alone allowed a unique designation of the animals into the two species classes.

2. CP bursts from unstimulated *H. pirardi* had the following characteristic features: (a) both positive and negative phases of the CP amplitudes increased progressively with successive pulses in a burst; (b) intervals were shorter in mid-burst than at the start or end of the burst; and (c) intervals progressively decrease in length with successive position in an inter-burst interval.

3. The CP pattern of *H. pseudoligactis* can be changed from one primarily of single CP's to one consisting predominantly of bursts of pulses by the following external stimulation: (a) increased electrode suction; (b) intermittant light stimulation; and (c) exposure to feeding stimuli. The three features of CP bursts found in unstimulated *H. pirardi*, are exhibited by the CP burst pattern induced by such stimuli in *H. pseudoligactis*.

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